REMARKS

In the Final Action dated January 19, 2005, claims 1-18 and 24-35 are pending and under consideration. Claims 1-7, 10-18, 24-30 and 33-35 are rejected under 35 U.S.C. §103(a) as unpatentable over Kamb (U.S. Patent No. 5,869,242) in view of Koster (U.S. Patent No. 6,500,621). Claims 1-7, 10-18, 24-30 and 33-35 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Kamb in view of Koster and further in view of McCarthy (WO 97/03210). Claims 8, 9, 31 and 32 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Kamb in view of Koster, or Kamb in view of Koster and further in view of McCarthy, each further in view of Vestal (U.S. Patent No. 6.057,543).

This response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

The Examiner has rejected the claims as allegedly obvious based on Kamb (U.S. Patent No. 5,869,242) in combination with one or more secondary references. Before addressing the Examiner's rejections, it is helpful to explain and highlight the key features of the present invention.

The present invention is predicated, in part, on the determination that a combination of single-base-specific cleavage and MALDI-TOF MS can be used directly to identify a difference of one or more nucleotides between nucleic acids. The present inventors realized that a method based on a combination of single-base-specific cleavage and MALDI-TOF MS would be useful in identifying mutations and polymorphisms in genomic DNA. Such a method does not require prior knowledge of the mutation to be detected, or pre-selection of a cleavage agent. In addition, the method is applicable to large nucleic acid molecules. Specifically, as the length

of a nucleic acid increases, the probability of obtaining fragments of identical nucleotide composition but different sequence also increases, thereby making detection of mutations and polymorphisms in a molecule more difficult. The present method has overcome this problem by combining MALDI-TOF MS with further separation such as post source decay (PSD) to distinguish between fragments having identical nucleotide compositions but different sequences of nucleotides. By employing computer simulation methods, the presently claimed method is able to determine more than 99% of all substitutions made in a 1000bp molecule subjected to base-specific cleavage and analyzed with MALDI-TOF-MS and PSD.

The presently claimed method therefore represents an improvement over methods disclosed in the references cited by the Examiner, whether considered individually or in combination. At most, the combined teachings of Kamb, McCarthy, Koster and Vestal extend to the use of MALD-TOF MS and PSD to either sequence nucleic acid fragments, or alternatively, analyze the products of a mutation detection procedure in which one or more enzymes are chosen to selectively cleave one of two sequences to be distinguished in order to amplify the size/mass difference between the two fragments. In contrast, in the present method, single-base-specific cleavage is used to generate fragments which are small enough to be analyzed by MALDI-TOF MS in order to detect differences in the composition of fragments. The claimed method is advantageous over the methods disclosed in the cited references in that no prior knowledge of the mutation is required; no selection of enzymes is required; and with further separation (such as PSD), the present method is effective in analyzing long nucleic acid molecules, for example, nucleic acid molecules of 250 bp or longer, as illustrated on page 29 of the specification.

Claims 1-7, 10-18, 24-30 and 33-35 are rejected under 35 U.S.C. §103(a) as unpatentable over Kamb (U.S. Patent No. 5,869,242) in view of Koster (U.S. Patent No. 6,500,621).

The Examiner contends that Kamb teaches a method for detecting a mutation by digesting a sample nucleic acid by using any art conventional technique and analyzing the mixture of digestion products by MALDI MS. The Examiner indicates that Kamb teaches that the digestion can be achieved with a restriction endonuclease, ribonuclease T1, DNase I, or uracil-N-glycosylase.

The Examiner admits that Kamb does not specifically teach analyzing the oligonucleotides using MALDI-TOF MS. However, the Examiner contends that Koster teaches methods for detecting the presence of a mutation or polymorphism by cleaving a nucleic acid with one or specific endonucleases to form a mixture of fragments and analyzing the fragments using MALDI-TOF MS (see column 18, lines 54-58).

The Examiner also admits that Kamb does not specifically teach a computer program to control the method of detecting a difference in one or more nucleotides of a sample nucleic acid as compared to a reference nucleic acid. However, the Examiner contends that the use of computer programs to control methods and store data obtained from nucleic acid analysis, particularly MALDI MS analysis, was conventional in the art at the time the invention was made.

Applicants previously submitted that Kamb's method of detecting a mutation is limited to instances where prior knowledge of the mutation is available. The Examiner argues in the Final Action that the teachings of Kamb are not limited to methods which require cleavage at the point of a mutation, citing the use of ribonuclease T1 and DNase I in Kamb. Further, during

a telephone interview conducted on April 12, 2005, the Examiner states that as ribonuclease T1 does not cleave at only a site containing a particular mutation, the analysis of the digestion products in Kamb allows for the detection of both known and unknown mutations. Moreover, the Examiner stated during the interview that the specification does not define "mutations at unknown locations" in a manner that would distinguish the claimed invention over the method of Kamb. In this connection, the Examiner states that the claimed methods also contemplate the use of PCR primers to amplify the nucleic acid molecule to be analyzed, as characterized in claim 2. Additionally, the Examiner indicates that the claims do not recite a length limitation for the fragment to be digested and then subjected to MALDI-TOF MS analysis.

Applicants respectfully submit that Kamb recognizes the problem that MALDI MS does not detect a difference of 9 Daltons in mass between oligonucleotide fragments of 30 base pair in length. See col. 6, lines 8-15 of Kamb. To address this problem, Kamb essentially provides a solution that is premised on knowledge of the mutation to develop differences that can be detected. Specifically, oligonucleotide fragments are subjected to cleavage at the point of the mutation in order to distinguish the mutated fragment from the wild type fragment. Additionally, because MALDI MS requires small fragments for proper analysis, Kamb selected primers to generate amplified products small enough, e.g., about 40 bp surrounding the site of an expected mutation for analysis by MALDI MS. Such selection of primers would also require knowledge of the location of the mutation. As admitted by Kamb, the method described by Kamb is directed to a region with "a known mutation" (col. 2, lines 49-50; col. 5, lines 24-25). Kamb does not teach how to detect a mutation in a large nucleic acid molecule where there is no information of the location or nature of the mutation. In contrast, as disclosed in the present specification (e.g., on page 12, lines 1-2), the present method does not require prior knowledge

of the mutation. Therefore, Applicants respectfully submit that Kamb does not provide adequate teaching to serve as a primary prior art reference relative to the claimed invention.

Further, as the Examiner has admitted, Kamb does not specifically teach analyzing the oligonucleotides using MALDI-TOF MS. In addition, Applicants respectfully submit that Kamb provides no motivation to those skilled in the art to combine base-specific cleavage and MALDI-TOF MS to distinguish a difference of one or more nucleotides between nucleotide molecules to be tested. Kamb has avoided the problem of the lack of sensitivity of MALDI MS by amplifying the difference between nucleotides to be detected (by cleavage at the point of the mutation) and has therefore no requirement for MALD-TOF MS or PSD. By providing this solution, Kamb in fact teaches away from the claimed method.

With respect to Koster, this reference appears to teach the use of the more sensitive MALDI-TOF MS in detecting point mutations and small deletions as well as small insertions in amplified DNA. However, Applicants respectfully submit that the method disclosed in Koster does not *directly* distinguish, through MALDI-TOF MS, between oligonucleotides that differ in length by one nucleotide. Instead, Koster discloses a complex strategy for detecting a mutation by employing MALDI-TOF MS. A detection primer complimentary to a region downstream of the mutated region is applied to the target strand. The primer is extended by a polymerase in a sequencing reaction using three dNTPs and a fourth NTP in dideoxy form (ddNTP). The primer is extended through the mutated region until the first ddNTP is incorporated. The mass of the extension products determined in MALDI-TOF MS defines the composition at the variable site.

Accordingly, even had those skilled in the art followed the teaching of Kamb and attempted to use mass spectrometry to compare fragments in order to detect a single substitution therein and had found that MALDI MS alone was insufficient, those skilled in the art would not

have found any motivation from Koster to use MALDI-TOF MS in place of MALDI MS to directly distinguish a difference of one or more nucleotides between nucleic acid molecules to be tested.

Applicants respectfully submit that the Examiner's determination that those skilled in the art would have been motivated to combine the teaching of Kamb and Koster is based on a hindsight construction. Applicants respectfully submit that the rejection of claimed subject matter under 35 U.S.C. §103 in view of a combination of prior art references requires that the suggestion to carry out the claimed invention must be found in the prior art, *not in Applicant's disclosure*. In re Vaeck, 947 F.2d 488, 492, 20 U.S.P.Q. 1438, 1442 (Fed. Cir. 1991).

The Examiner further indicates that Applicants have not addressed the rejection with respect to claims 10-18 and 34, directed to a computer program which controls a method for detecting a mutation.

In an effort to favorably advance prosecution of the present application, Applicants have canceled these claims without prejudice.

In view of the foregoing, it is respectfully submitted that the based on Kamb in view of Koster, is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-7, 10-18, 24-30 and 33-35 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Kamb in view of Koster and further in view of McCarthy (WO 97/03210). The Examiner indicates that the rejection is based on the interpretation that the claims encompass methods in which uracil-N-glycosylase is used to directly detect the presence of a mutation and cleave the sample nucleic acid at a site of nucleotide variation.

The Examiner admits that Kamb does not specifically teach using uracil-N-glycosidase to directly detect the presence of a mutation that alters a cleavage site. However, the

Examiner contends that McCarthy teaches a method for detecting a mutation in a nucleic acid. According to the Examiner, McCarthy specifically teaches that uracil-N-glycosylase can be used to specifically detect the presence or absence of a T residue at a specific location in a nucleic acid by generating an amplified product containing dUTP. Therefore, it is the Examiner's opinion that those skilled in the art would have been motivated to use uracil-N-glycosylase in the method disclosed by Kamb to achieve effective analysis of DNA samples for detecting a mutation involving thymine.

In the first instance, Applicants respectfully submit that Kamb does not provide sufficient teaching to serve as a primary reference with respect to the presently claimed invention. As submitted above, Kamb does not teach how to detect a mutation within a nucleic acid of at least 250bp in length, where there is no prior information on the location or nature of the mutation. Moreover, neither Kamb nor Koster provides any motivation to combine the respective teachings in order to detect an unknow mutation by utilizing MALDI-TOF MS.

Applicants respectfully submit that the motivation to combine the teaching of Kamb and McCarthy is not found in any of the cited references. Specifically, McCarthy does not provide any teaching or suggestion for the use of uracil-N-glycosylase in cleaving nucleic acid molecules to produce small fragments for analysis by MALDI-TOF MS alone or in conjunction with further separation techniques to distinguish between fragments of identical composition but different sequence.

Therefore, it is respectfully submitted that the rejection based on Kamb in view of Koster and further in view of McCarthy, is improper. Withdrawal of the rejection is therefore respectfully requested.

Claims 8, 9, 31 and 32 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Kamb in view of Koster, or Kamb in view of Koster and further in view of McCarthy, each further in view of Vestal (U.S. Patent No. 6.057,543).

The Examiner admits that the combined references, i.e., Kamb, Koster and McCarthy, do not teach further separation of the DNA fragments by post source decay (PSD). However, the Examiner contends that Vestal teaches the use of PSD in combination with MALDI TOF MS to analyze nucleic acids. Therefore, the Examiner concludes that it would have been obvious to one of ordinary skill in the art to have modified the method of Kamb so as to have further separated the oligonucleotide fragments by PSD in order to improve the resolution and accuracy of the detection method and to allow for further analysis of the molecular structure of the fragments, as suggested by Vestal.

Applicants respectfully submit that none of the cited references provide any motivation that would have led those skilled in the art to attempt to arrive at the claimed method with a reasonable expectation of success. Specifically, none of the references provide any motivation to modify the methods disclosed in Kamb, McCarthy or Koster by following the disclosure of Vestal to incorporate a further separation step to improve the sensitivity of mutation detection. Applicants respectfully submit that the advantages of MALDI-TOF MS or PSD alone do not warrant a conclusion that those skilled in the art would have been motivated to modify the methods disclosed, e.g., by Kamb.

Applicants further respectfully submit that when the fragment derived from the mutated sequence coincides with other fragments of identical nucleotide composition in the wild-type sequence it disappearance will go undetected. Similarly, the appearance of a new fragment in the mutated sequence will go unnoticed if it has an identical nucleotide composition to one or

other cleavage products. If both these conditions exist for all cleavage reactions then the mutation will be missed. The probability of this occurring increases with longer fragments and in accordance with the present invention this further problem is solved by the use of the second dimension such as post sauce decay. As the spectrum from the decay is dependent on the sequence of the oligonucleotide and not the nucleotide composition, the aforementioned limitation is bypassed, generating a method of mutation detective that is extremely sensitive for analysing longer fragments (the fragments analysed are still tiny).

In this regard, Applicants reassert that Kamb's teaching applies particularly to detecting mutations within a known region, therefore the analysis is directed to small PCR fragments (about 40 bp) surrounding the location of an expected mutation. Smaller nucleic acids are less likely to produce oligonucleotide fragments having the same size but different compositions. Therefore, even assuming that those skilled in the art were to follow the teaching of Kamb, there would have been no motivation to modify the teaching of Kamb to include a further separation.

Applicants further respectfully submit that the method provided by the present application has achieved unexpected and superior results relative to the methods disclosed in the cited references. As described in the specification, on page 29, the inventors determined the sensitivity of the method by developing a computer program which simulated the method, processing 100 Kb of random coding sequence from a gene database. The expected number of base substitutions that would be identified when comparing two homozygous mutations over a 250 base pair PCR distance was determined to be 98.5%. The comparable figure is 95% when a homozygous is compared to a heterozygous mutation. If each mass peak from a base specific cleavage is analyzed using a secondary dissociation technique such as post sauce decay on the

MALDI-TOF machine, then sensitivity of mutation detection improves dramatically. This has also been simulated and for a 1000 base pair fragment subjected to base specific cleavage, and analyzed with PSD, 99% of all substitutions would be detected for a homozygous to heterozygous comparison and 99.8% when two homozygous are compared.

In view of the foregoing, Applicants respectfully submit that the rejection based on the combination of Kamb, Koster, McCarthy, and Vestal, is overcome. Withdrawal of the rejection is therefore respectfully requested.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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